4. ZOONOTIC AND PARASITIC INFECTIONS: CLINICAL, EPIDEMIOLOGICAL AND LABORATORY ASPECTS

4.1 PCR ANALYSIS IN THE REAL TIME REGIMEN AS A LONG-TERM METHOD FOR LABORATORY DIAGNOSIS OF RICKETTSIOSIS


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The actuality of studying the natural foci of rickettsiosis and the expediency of researches for the revealing of rickettsia DNA in ticks of the Crimea are caused by peculiarities of the region that are favorable for the circulation and preservation of pathogens in the nature.

The purpose of the study was to define the contamination of ticks by rickettsia and determine their species belonging.

Tasks: the organization of ticks collection and carrying out of their specific identification; carrying out of the laboratory researches of ticks — PCR — analysis in the real time regimen (PCR-RV)

Materials and methods — epidemiological and literary data on the study of rickettsiosis in the Crimea; parasitological methods (collection of ticks for the standard flag and dragging, manual collection from animals), specific identification of the ticks, laboratory methods (revealing of rickettsia DNA by PCR-RV using reagents set “RealBest DNA Rickettsia species” (“Vector-Best”, Novosibirsk).

1342 specimens of ticks are collected from August to October 2016 and analyzed in total. Specific composition is presented by: Rhipicephalus sanguineus — 65.3%, Dermacentor marginatus — 21.8%, Hyalomma marginatum — 9.5% and Dermacentor marginatus — 3.4%.

Using the PCR test “RealBest DNA Rickettsia species” in 470 from 1342 nucleic acid samples isolated from individual ticks suspensions, DNA marker of rickettsia was detected. 114 positive samples of rickettsia DNA were selected for additional amplicons production and sequencing of their sequences by 3–4 genes (gltA, ompA, ompB and sca4).

The diagnosis was confirmed by a coprological examination of feces for lamblia cysts.

In recent years, more and more often among residents of the Russian Federation, especially among children, cases of parasitic infestations have been recorded, among which a special place is occupied by lambliasis, which often occurs under the mask of the lesion of gastrointestinal tract and is not always recognized in time.

The purpose of the study was to analyze lesions of the gastrointestinal tract in schoolchildren invaded by lamblia. Under supervision there were 55 children of school age of whom 60% were children with gastrointestinal lesions. The diagnosis was confirmed by a coprological examination of feces for lamblia cysts.

According to the results of ultrasound investigation, all children showed lesions of the gastrointestinal tract, manifested in the form of reactive changes in the pancreas — 2.1%, reactive changes in the liver — 15.2%, signs of biliary dyskinesia — 18.2%, combined liver and pancreatic lesions — 15.2%, combined liver and pancreas damage, and signs of biliary dyskinesia — 18.2%, liver damage and signs of biliary dyskinesia — 12.1%, as well as pancreatic lesions and signs of dyskinesia of bile ducts — 9.1%. In most cases — 75.8% of children received the drug Makmiror at the rate of 15–30 mg per 1 kg of body weight for 7 days. Albendazole was received by 24.2% of children at 12 mg/ kg body weight.

Lambliasis was registered most often in children of primary school age, which may indicate an incomplete knowledge of the rules of personal hygiene. The main causes of the disease were non-compliance with personal hygiene and contact with domestic animals, more often with cat. The main complaints of children were abdominal pain, nausea, decreased appetite, loosening of the stool and allergic reactions to the skin.
The purpose of the work is to develop universal recommendations for clinicians for the management of patients with tick-borne borreliosis.

Tasks of the study was to compare the distinctive features of epidemiological anamnesis, clinical manifestations, indicators of laboratory and instrumental diagnostics, and criteria of dispensary registration of patients with tick-borne borreliosis of erythema and non-erythema form.

We have analyzed about 34 patients from the maps of municipal institutions in Ulyanovsk. Forms of borreliosis were divided evenly into erythemic and non-erythemic forms in 17 patients (50%).

In the first 7 days 9 (26%) patients addressed, on 8—14 days — 5 (15%), on 15—30 days — 4 (12%) and 16 (47%) arrived at a later date. The complaint in 100% was the presence of itching and in 50% of erythema, which was accompanied by subjective sensations (burning sensation or compaction — 17 (50%), increase — 9 (26%) patients. In 3 (8%) patients complications with the defeat of the musculoskeletal system (rheumatoid arthritis) were revealed. Serological diagnosis (ELISA) was performed in 17 (50%) patients. Antibodies were found in 14 (41%), IgM levels ranged from 0.470 to 0.633. Terms of appearance were different (15—43 days). In 3 (9%) people the level was below normal. The remaining half of the patients were not examined for various reasons. Clinical and electrocardiographic manifestations of dysfunction of the circulatory system were noted in non-erythematous form (50%).

Serological diagnosis of tick-borne borreliosis by ELISA, due to the late appearance of antibodies in the early stages of little informative, which necessitates the introduction of modern rapid methods. Patients with non-erythematous form of tick-borne borreliosis require more attention and detailed laboratory and instrumental diagnosis, as there is a risk of complications from vital organs and systems.

**4.4 DETECTION OF GENETIC MARKERS OF TICK-BORNE RICKETTIOsis WITH THE PCR**

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Leptospirosis is found all over the world both in humans and in many species of agricultural, domestic and wild animals. The disease caused by individual serovars of the pathogen is characterized by a severe clinic and high mortality. Leptospira grow very slowly and only on special nutrient media. Together with the difficult pathogen isolation there is also the problem of its identification. According to the modern genosystematics several molecular biology methods were proposed to determine the Leptospira species. Mass-spectrometry direct profiling of proteins is easy to set up and widely used to diagnose most bacterial infections, while the available databases of Leptospira spectra are absent.

The aim of this study was the development of a protein spectra database for identification of the Leptospira species.

Our database contains information about 28 Leptospira reference strains of 28 serovars including eight most common species L. interrogans, L. borgpetersenii, L. interrogans serovar Pomona, Tarassovi, Australis, Sejroe, Autumnalis, Bataviae, Ballum, Pyrogenes, Javanica, Hebdomadis, Louisiana, Panama, Lyme, Sarmin, Djasiman, Mini, Minham, Sema-